17. (Amended) The composition of claim 14 [or 15] wherein at least one of the cryoprotective agents is arabinogalactan.

19. (Amended) The composition of claim [18] 14 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.

20. (Amended) The composition of claim 14 [or 15] further comprising a cryoprotective agent other than arabinogalactan or a biological or functional equivalent thereof which does not penetrate the cell membrane.

21. (Amended) The composition of claim 14 [or 15] which does not comprise protein.

22. (Amended) The composition of claim 14 [or 15] which is infusible.

23. (Amended) The composition of claim 14 [or 15] which does not comprise dimethylsulfoxide.

24. (Amended) The composition of claim 14 [or 15] wherein the cells are human cells.

36. (Amended) The composition of claim 14[, 15] or 31 wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.

Remarks

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks herein, is respectfully requested. Claims 14, 16-17, 19-24 and 36 are amended, and claims 15 and 18 canceled. Claims 1-12, 14, 16-17, 19-24, and 26-36 are pending.

The Examiner rejected claims 1-12, 14-24 and 31-36 under 35 U.S.C. § 102(b) as anticipated by, or in the alternative under 35 U.S.C. § 103(a) as obvious over, the LAREX

Material Safety Data Sheet (which as referred to herein includes the LAREX Technical Data Sheet) or WO 97/35472. The Examiner also rejected claims 1-12, 14-24 and 26-36 under 35 U.S.C. § 102(a) as anticipated by, or in the alternative under 35 U.S.C. § 103(a) as obvious over, WO 97/35472. These rejections are respectfully traversed.

The LAREX Material Safety Data sheet relates that Cellsep™ powder contains at least 99% arabinogalactan (AG), and that AG is approved as a food additive by the FDA. It is also disclosed that Cellsep™ powder is a medium for density gradient cell separation which provides superior resolution of a wide variety of cell types and cellular organelles. It is further disclosed that Cellsep™ isotonic solutions are available for lymphocytes and platelets. No mention is made in the LAREX Material Safety Data Sheet of AG-containing cryopreservation solutions for certain hematopoietic cells, e.g., freshly isolated lymphocytes, stem cells, or lymphocytes which are modified *ex vivo*, or methods to cryopreserve those cells.

WO 97/35472 relates to the use of AG in cryopreservation media for immortalized mammalian somatic cells. Although WO 97/35472 indicates that the described media may be employed with a variety of cell types including human cells (page 5, line 2) and blood cells (page 10, line 4), the only data provided in the WO 97/35478 specification is for seven lines of immortalized mammalian cells (page 13). These included three lines derived from rodent epithelial cells, a line derived from mink fibroblasts, a line derived from human fibroblasts, a line derived from bovine endothelial cells (CPAE cells), and a line derived from murine pre-neoplastic mammary cells. Thus, no blood-derived hematopoietic cells are represented in the seven lines of cells disclosed in WO 97/35472

These seven lines were frozen in 6 different media (Table 1). For media containing AG, it is disclosed that AG was prepared as a 50% w/v concentrated stock dissolved in a buffered isotonic salt solution. This stock was used directly (medium 3, i.e., 50% AG) or in combination with other components. Medium 4 has 20% AG and 10% DMSO; medium 6 has 15% AG and 20% serum, medium 2 has 10% AG and 20% DMSO; and medium 5 has 10% AG, 10% DMSO and 20% serum. Medium 1 has 10% DMSO and 20% serum (no AG). Note that media which includes DMSO or serum is not generally suitable for administration to a human (claim 14) due to DMSO-related toxicity or the potential for a transmissible infectious agent in serum.

With respect to immediate post-thaw viability for all cell types tested, it is disclosed that there was no difference in post-thaw viability for 4 of the media relative to "the industry standard" (cell culture medium + serum + DMSO) (page 14), however, cells frozen in media with AG and serum had reduced viability. It is also noted that there was "substantially no difference" in plating efficiency at day 1 for 6/7 of the cell types (page 14). At six days post-thaw, it is disclosed that there was "substantially no difference" between treatment groups (page 15). Table 2 shows the ranking of the media with respect to growth rates (Day 6/Day 1) for CPAE cells (media 3 > media 5 > media 2 > media 1 > media 4 > media 6). WO 97/35472 concludes that AG "can be used to replace serum in a standard freezing medium, in a formulation with DMSO, for all cell types studied" (page 15, emphasis added) and that freezing in 50% w/v AG was better or equivalent to the standard media for 5/7 cell types tested (page 15).

WO 97/35472 generally discloses that the cells may be cooled or frozen during storage to about or below 4°C, for example to about -200°C. An exemplary freezing procedure is described as resuspending cells in an AG-containing freezing medium (1×10^6 - 1×10^7 cells/vial), aliquoted into 1.8 ml cryovials, equilibrated for about 30 minutes at 4°C, step-cooled for 18 hours at -80°C and immediately transferred to liquid nitrogen (-196°C) (page 8 and Example 2).

Nevertheless, methods and compositions useful to cryopreserve one cell type are not necessarily the same as the methods and compositions employed for other cell types, as each cell type has different biological and physical properties. In this regard, the Examiner is requested to consider the Rule 132 Declaration enclosed herewith, which is executed by Dr. Allison Hubel, the inventor of subject matter claimed in the above-identified application. In the Declaration, Dr. Hubel states that a variety of interrelated factors influence the ability of cells to survive the stresses of freezing and thawing including (1) the composition of the cryopreservation solution; (2) the temperature history of the sample during cooling (e.g., cooling rate); and (3) the biological and biophysical characteristics of the cell/tissue being frozen (paragraph 5 of the Declaration). Dr. Hubel also states that during rapid cooling, there is insufficient time for water to leave the cell in response to the increase in extracellular solution concentration resulting from the removal of water experienced during freezing (paragraph 7 of the Declaration). Undercooling of the cell relative to the extracellular solution results in intracellular ice formation,

a lethal event, and slow cooling can result in excessive dehydration of the cell that is also damaging to the cell (paragraph 7 of the Declaration). Dr. Hubel also states that the relative water content of a cell during freezing is a function of the cell type (with each cell type exhibiting its own unique biophysical characteristics) and the function of the solution composition in which the cell is suspended (paragraph 7 of the Declaration). Evidence that survival and cooling rate vary with the composition, and that different cell types have different cooling rates when present in the same freezing medium, is provided in paragraphs 8 and 9 of the Declaration.

In this regard, the Examiner is also requested to consider page 97 of Sputtek et al. (In: Clinical Applications in Cryobiology, CRC Press, 1991), where it is noted that the conditions employed to freeze red blood cells do not result in viable white blood cells (a copy is provided herewith). Further, in Hubel (Transfusion Med. Rev., 11, 224 (1997)) (a copy is provided herewith), it is disclosed that the membrane permeability parameters for a number of blood cell types including lymphocytes was found to be distinctive (see Table 1). In addition, Figure 3 in Hubel provides data showing that freshly isolated $CD34^+$ cells and cultured, transduced $CD34^+$ cells have different physical characteristics at different temperatures, including water permeability, cell volume and the osmotically inactive cell volume fraction (page 228).

Yet further evidence that different cell types have different properties in any particular cryopresentation medium is shown in Table 3 and 4 of Applicant's specification. Tables 3 and 4 show the differences in cell recovery for activated peripheral blood lymphocytes versus cultured peripheral blood lymphocytes and genetically altered peripheral blood lymphocytes versus normal peripheral blood lymphocytes in the same AG-containing cryopreservation medium and relative to DMSO-containing medium.

Because the concentration of AG useful in a cryopreservation medium is based on the biophysical properties of each cell type, and so varies with cell type, Dr. Hubel concludes that the disclosure in the LAREX Material Data Safety Sheet and in WO 97/35272 does not enable a cryopreservation composition for freshly isolated lymphocytes, stem cells or lymphocytes which are modified *ex vivo* or a method to cryopreserve those cells. Thus, neither the LAREX Material Safety Data Sheet or WO 97/35472 anticipates or renders obvious Applicant's invention.

Therefore, the Examiner is respectfully requested to withdraw the § 102(b), § 102(a) and

§ 103(a) rejections of the claims.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6959) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

ALLISON HUBEL,

By her Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6959

Date August 27, 2001 By _____
Janet E. Embretson
Reg. No. 39,665

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Box AF, Commissioner of Patents, Washington, D.C. 20231, on this 27th day of August, 2001.

Dawn M. Poole
Name

Dawn M. Poole
Signature